inducing conditions are *in vivo*.

REMARKS

Claims 26, 27, 32-37, and 39-62 are pending in this application. These claims are directed to methods for transplanting CNS neural stem cell progeny to a host. Applicants have amended claims 34 and 61-62 in accordance with the Examiner's suggestions to obviate the 35 U.S.C. § 112, second paragraph, rejections. These amendments add no new matter.

The central nervous system (CNS) neural stem cells and the various methods and uses of these cells claimed in this application and related counterparts are pioneering inventions. The inventors have been widely recognized by the scientific community as the first to identify and isolate CNS neural stem cell cultures, and to teach methods for proliferating and differentiating those cell cultures, as well as various methods and systems using those cultures (such as the transplantation methods claimed here).

The subject matter of the present application, uses of *in vitro* CNS neural stem cell compositions, has been now been pending since 1991. The present application claims priority back to United States patent application 07/726,812, filed July 8, 1991, now abandoned. The application also claims priority to United States patent application 07/961,813, filed Oct. 16, 1992; United States patent application 07/967,622, filed Oct. 28, 1992; and United States patent application 08/010,829, filed Jan. 29, 1993, all now abandoned.

This patent application is one of several related co-pending applications that are based on the same specification. Of those applications, three have issued as United States patents. United States patent application 08/483,122 has issued as United States patent 5,750,376 (claims directed to genetically modified central nervous system neural stem cell cultures). United States patent application 08/486,648 (claims directed to central nervous system neural stem cell cultures) has issued as United States patent 5,851,832. United States patent application 08/486,307 (claims directed to growth factor-induced proliferation of neural precursor cells *in vivo*) has issued as United States patent 5,980,885. All three of these applications contain a specification identical to the above-identified application. All three have the same filing date as the above-identified application.

122

THE § 112, SECOND PARAGRAPH REJECTION

The Examiner has rejected claims 34, 61, and 62 under 35 U.S.C. § 112, second paragraph, alleging that the claims are indefinite. The Examiner has suggested phrasing that would overcome these rejections. Applicants thank the Examiner for these suggestions and amend claim 34 to recite proper Markush language. Applicants have also amended claims 61 and 62 to recite a method. The rejections under 35 U.S.C. § 112, second paragraph, are now moot and should be withdrawn.

THE § 112, FIRST PARAGRAPH REJECTION

The Examiner has rejected claims 26, 27, 32-37 and 39-59 under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. The Examiner alleges specification teaches that "the" use for the transplant method is to produce a therapeutic effect in the host and that the transplantation of multipotent neural stem cell progeny into a host has not been demonstrated to provide any therapeutic benefit to the host. Applicants respectfully traverse.

The claims do ***not*** recite a therapeutic treatment. The claims recite a method of transplanting CNS neural stem cell cultures to a host, whether for non-therapeutic or therapeutic uses. The specification teaches ***both*** a non-therapeutic use for transplanting neural stem cells ***and*** therapeutic uses for transplanting neural stem cells (*see below*).

One skilled in the neurobiological art could, at the date of filing, have readily transplanted the CNS neural stem cells of the invention into a host, *without undue experimentation*. *See,* MPEP § 2164.01 "Test of Enablement". The CNS neural stem cells of the invention are a strikingly useful source of transplantable neural tissue, as noted in the specification:

> The inability in the prior art of the transplant to fully integrate into the host tissue, and the lack of availability of cells in unlimited amounts from a reliable source for grafting are, perhaps, the greatest limitations of neurotransplantation. It would be more preferable to have a well-defined, reproducible source of neural tissue for transplantation that is available in unlimited amounts.

See, specification, pg. 11, lines 15-20. Because CNS neural stem cells can be cultured *in vitro*, and are self-renewing, multipotent, and capable of differentiating to a variety of desirable cell

types depending upon culture conditions or tissue environment, neural transplantation using the cells of the invention is greatly facilitated compared to prior art transplantation methods.

The specification provides ample guidance as to how to transplant CNS neural stem cells (*see,* specification, pg. 36, line 10, to pg. 42, line 13; pg. 68, line 16, to pg. 69, line 18; pg. 78, line 17, to pg. 71, line 6; pg. 96, line 12, to pg. 97, line 28). The specification also teaches that following transplantation, the CNS neural stem cells differentiate to various cell types appropriate to the tissue into which the CNS neural stem cells are transplanted (*see,* specification, pg. 69, line 19, to pg. 70, line 7; pg. 97, line 29, to pg. 103, line 14). The prior art had taught methods of transplantation (*see,* specification, pg. 6, line 19, to pg. 11, line 12), although none of the prior art taught or could have taught the claimed transplantation of the CNS neural stem cells of the invention.

The nature of the invention is such that the transplantation of CNS neural stem cells can be readily verified (*see,* specification, pg. 71, line 26, to pg. 72, line16; pg. 73, lines 4-30). The transplantation methods of the invention were well within the abilities of one of skill in the art, as shown by the voluminous evidence, subsequently produced by Applicants and by other neurobiologists, that the CNS neural stem cells of the can be transplanted to a host and that following transplantation, the CNS neural stem cells differentiate, in the appropriate tissues conditions, to important and useful cell types.

In summary, the specification enables one skilled in the neurobiological art to transplanted the CNS neural stem cells of the invention into a host, for either a non-therapeutic use or a therapeutic use, without undue experimentation.

Non-Therapeutic Use.

The Examiner's determination that the claims are not enabled for the non-therapeutic use is "without observance of procedure required by law." Administrative Procedure Act (APA), 5 U.S.C. § 706(2)(D). As shown below, the rejection fails to follow the procedures of the *Manual of Patent Examining Procedure* (MPEP). The MPEP is made available to the public and has been held to describe procedures on which the public can rely. *In re Kaghan*, 387 F.2d 398, 401, 156 USPQ 130, 132 (CCPA 1967). The MPEP does have significant legal effect. *See, e.g., Ethicon* , 849 F.2d at 1425; 7 USPQ2d 1152 (1988); *Paperless Accounting, Inc. v. Bay Area Rapid Transit*

Sys., 804 F.2d 659, 663, 231 USPQ 649 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 933 (1987); *Patlex Corp. v. Mossinghoff*, 758 F.2d 594, 606, 225 USPQ 243, 252, *modified*, 771 F.2d 480, 226 USPQ 985 (Fed. Cir. 1985). The Rules of Practice in the Patent Office, when not inconsistent with the statutes from which they are derived, have the force and effect of law. *In re Hession, Jr.*, 49 CCPA 809, 296 F.2d 930, 132 USPQ 40 ; *Land v. Dreyer*, 33 CCPA 1108, 155 F.2d 383, 69 USPQ 602, *In re Newton*, 163 USPQ 34 (1969).

The MPEP § 2164.01(c) "How to Use the Claimed Invention" describes how a Patent Examiner must address issues of enablement:

> In contrast, when a compound or composition claim is not limited by a recited use, any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use. If multiple uses for claimed compounds or compositions are disclosed in the application, then an enablement rejection must include an explanation, sufficiently supported by the evidence, why the specification fails to enable each disclosed use. In other words, if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention.

Here, the specification teaches a ***non-therapeutic*** transplantation utility by teaching that transformed CNS neural stem cells transplanted into a host produce β-galactosidase in the host. An Examiner had earlier acknowledged that the specification "exemplifies the implantation of multipotent neural stem cell progeny into animal models" (Office action of January 21, 1999, Paper 29, pp. 3-4). That Examiner had also acknowledged that these CNS neural stem cells survive this transplantation. That Examiner had further acknowledged that transformed CNS neural stem cells transplanted into a host produce β-galactosidase in the host (thereby providing the non-therapeutic utility of determining neural development events).

In the October 21, 1999, Office action, however, the Examiner now alleges that the specification does not assert any such non-therapeutic utility (Office action of October 12, 1999, Paper 36, pg. 3, para. 4). The Examiner alleges that the specification pg. 78, Example 27 does not involve the transplantation of neural stem cells, but rather only involves the administration of a recombinant retrovirus and several growth factors. The Examiner overlooks, however, the disclosure of the specification, pp. 72-74, EXAMPLE 20 and pp. 96-101, EXAMPLE 45, which provide working examples of transplantation of neural stem cells for the ***non-therapeutic*** use of

determining neural developmental events, as described in the specification, pg. 78, Example 27, *citing,* Walsh & Cepko, 241 Science 1342:

> <u>*EXAMPLE 20: Proliferation of Neural Stem Cells from Transgenic Mice.*</u>
>
> Transgenic mice were produced using standard pronuclear injection of the MBP-*lac*Z chimeric gene, in which the promoter for MBP directs the expression of *E.* coli β-galactosidase *(lacZ).* Transgenic animals were identified by PCR using oligonucleotides specific for *lac*Z.
>
> Neurospheres were prepared from E15 transgenic mice and DNA negative littermates using the procedures set forth . . .
>
> The neural stem cells derived from the transgenic animals were indistinguishable from non transgenic stem cells in their potential for differentiation into neurons, astrocytes, and oligodendrocytes. The MBP promoter directed the expression of the β-galactosidase reporter gene in a cell-specific and developmentally appropriate fashion. The transgene expression is highly stable as oligodendrocytes derived from late passage MBP-*lac*Z neurospheres (20 passages), expressed the β-galactosidase gene. Thus, ***transgenically marked neurospheres are likely to be an excellent source of cells for glial cell transplantation***.

See, specification, pp. 72-74 (***emphasis added***).

> <u>*Example 45: Transplantation of Multipotent*</u>
> <u>*Neural Stem Cell Progeny in Animal Models*</u>
>
> I. TRANSPLANTATION PROCEDURE
>
> **1. Neurosphere preparation**
>
> Neural tissue was obtained from normal embryonic or adult CD 1 mice and from embryonic or adult Rosa 26 mice (transgenic animals derived from C57/BL/6 mice, which express the β-galactosidase gene in all cells, thus allowing the ***transplanted cells*** to be easily detected in host tissue). Neurospheres were generated using the procedures described . . .
>
> **2. Labeling and Preparation of Neural Stem Cell Progeny**
>
> 16 hours ***prior to transplantation***, neurospheres derived from embryonic and adult tissue were labeled with BrdU . . . The cells were then transferred to a microcentrifuge tube for storage on ice prior to ***transplantation***. When ready for use; cells were resuspended prior to each cell injection by drawing cells into an eppendorf pipette tip (200 or 1000 μl).

3. Transplantation of Neural Stem Cell Progeny

The donor neural ***stem cell progeny were transplanted*** into selected sites in the brain of normal, healthy neonate or adult CD1 or C57BL/6 mice or adult Wistar or Sprague-Dawley rats. In some cases, embryonic cells from CD1 mice received *in vitro* gene transfer procedures prior to ***transplantation of the cells***. .

4. Analysis of Transplanted Neural Stem Cell Progeny

The animals were allowed to survive for 2-12 weeks prior to sacrifice. . . Survival of ***transplanted cells labeled with fluorescent beads*** were identified by the localization of fluorescent beads within the cell cytoplasm. BrdU labeled cells (cells that had incorporated BrdU into their DNA during cell division in culture prior to ***transplantation***) were identified using antibodies . . . Antibodies . . . were then used to identify the ***differentiation of the transplanted cells. Cell transplants derived from transgenic animals*** expressing β-galactosidase were histochemically analyzed . . . and by immunohistochemical staining. For Rosa 26 cells, antibodies against β-galactosidase were used to identify ***the transplanted cells*** and antibodies to NeuN were used to identify cells that had differentiated into neurons. Human cells were identified with HLA antibodies . . .

The results obtained from the animal models described below are summarized in Tables II-V.

A. MODEL OF HUNTINGTON'S DISEASE

Rats . . . sustained an ibotenate lesion of the striatum, stimulating Huntington's Disease in the animals. 7 days after the lesion, the animals received an injection of [CNS neural stem] cells . . . Injected cells were labelled with fluorescein-labelled micrcispheres. Animals were given behavioral tests before the lesion, after the lesion, and at various intervals after the ***transplant*** to determine the functionality of the grafted cells at various postoperative time points, . . . Neuronal and glial phenotype was identified by dual labeling of the cells with antibody to NSE and GFAP.

B. PARKINSON'S DISEASE

Two animal models of Parkinson's Disease were used. In the first model, unilateral dopamine neurons of the substantia nigra were lesioned by the stereotaxic administration of 6-OHDA into the substantia nigra . . . In one series of experiments, multipotent neural stem cell progeny obtained from embryonic Rosa 26 mice, were prepared . . . The neural stem cell progeny were labeled, prepared, and ***transplanted into the striatum*** of the lesioned C57BL/6 mice . . .

In a second series of experiments, the [CNS neural stem] cells ***were administered to the same regions in the brains*** of adult 6-OHDA Wister rats. In a third series of experiments, proliferated fetal human [CNS neural stem] cells . . . ***were transplanted into the striatum*** of the 6-OHDA lesioned CD 1 mice. . .

> The second animal model used was the adult mutant Weaver mice . . and the proliferated progeny of multipotent neural stem cells derived from embryonic Rosa 26 mice were injected into the striatal region of the animals . . .
>
> C. <u>CARDIAC ARREST</u>
> Transient forebrain ischemia was induced in adult Wistar rats . . . The progeny of proliferated multipotent neural stem cells, derived from embryonic Rosa 26 mice, were . . ***transplanted into the striatal region*** of the ischemia lesioned rats. . . β-gal positive cells, indicating surviving cells from the Rosa 26 donor, were detected in the lesioned hippocampal region. In addition, double labeled β-gal/NeuN$^+$ cells were found indicating that ***transplanted*** cells had differentiated into neurons.

See, specification, pp. 96-101 (***emphasis added***).

In summary, the specification shows that a "use is enabled when multiple uses are disclosed, [and that] the application is enabling for the claimed invention" MPEP § 2164.01(c). Accordingly, Applicants respectfully request that this rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

<u>Therapeutic Use.</u>

As shown in the Amendment filed July 21, 1999 (Paper No. 35), Applicants have provided overwhelming evidence of record that demonstrates enablement of the claimed methods for transplantation of CNS neural stem cells. The Examiner alleges that specification must teach one skilled in the art not only how to perform the claimed method but also *how* to use the claimed method. ***It does.***

As shown in the Amendment filed July 21, 1999 (Paper No. 35), the specification provides ample guidance as to *how* to transplant CNS neural stem cells (*see,* specification, pg. 36, line 10, to pg. 42, line 13; pg. 68, line 16, to pg. 69, line 18; pg. 78, line 17, to pg. 71, line 6; pg. 96, line 12, to pg. 97, line 28). The specification teaches that following transplantation, the CNS neural stem cells differentiate to various cell types appropriate to the tissue into which the CNS neural stem cells are transplanted (*see,* specification, pg. 69, line 19, to pg. 70, line 7; pg. 97, line 29, to pg. 103, line 14). The specification provides further guidance as to *how* to use the claimed method. The transplantation of CNS neural stem cells can be readily verified (*see,* specification, pg. 71, line 26, to pg. 72, line16; pg. 73, lines 4-30).

The transplantation methods of the invention were well within the abilities of one of skill

in the art, as shown by the voluminous evidence, subsequently produced by Applicants and by other neurobiologists, that the CNS neural stem cells of the can be transplanted to a host and that following transplantation, the CNS neural stem cells differentiate, in the appropriate tissues conditions, to important and useful cell types.

<u>*Remyelination*</u>.

The Examiner alleges that "nowhere in the specification are any transplantation methods taught that result in a therapeutic effect." The Examiner is incorrect.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), the specification teaches and provides working examples of transplantation and remyelination. Patches of myelin were found in the dorsal columns of the spinal cord of the recipients of both rat and mouse cells (*see,* specification, pg. 69, line22, to pg. 70, line 7). The demonstration of partial remyelination is evidence of an enabled method of transplantation. Because remyelination can begin only ***after*** transplantation, the specification must therefore enable transplanting CNS neural stem cells to a host.

However, the Examiner alleges that the "evidence of remyelination" is not enabling because "the specification does not teach any benefit to using the method to produce partial remyelination." (Office action of October 12, 1999, paper 36,pg. 4, para. 1). Again, the Examiner is incorrect.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), the specification teaches that transplantation, followed by partial remyelination, is useful (*see,* specification, pg. 42, lines 9-13:

> It should also be borne in mind that in some circumstances remyelination by precursor cells will not result in permanent remyelination, and repeated injections will be required. Such therapeutic approaches offer advantage over leaving the condition untreated and may spare the recipient's life.

As also shown in the Amendment filed July 21, 1999 (Paper No. 35), Applicants have provided the Declaration under 37 C.F.R. § 1.132 by Dr. Joseph P. Hammang, in United States patent application 07/961,813, which shows that the "precursor cells [*i.e.,* CNS neural stem cells] can be harvested and transplanted into a myelin-deficient recipient wherein the precursor cells can differentiate into oligodendrocytes and remyelinate the axons of the recipient." This Declaration is also evidence for the enablement of CNS stem cell ***transplantation***.

As further shown in the Amendment filed July 21, 1999 (Paper No. 35), Applicants have provided the Declaration under 37 C.F.R. § 1.132 that had been submitted in co-pending application United States patent application 08/479,796, which also provides additional evidence of remyelination in rodents and dogs (*see*, Declaration, para. 11-30). These data are also evidence for the enablement of CNS stem cell ***transplantation***.

In summary, the specification enables ***how to use*** the claimed methods of transplantation of CNS neural stem cells for a therapeutic use, such as partial remyelination, and teaches that the therapeutic use of partial remyelination has a therapeutic benefit. Accordingly, Applicants respectfully request that this rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

Characteristics of CNS neural stem cells.

The Examiner alleges that while "the specification teaches several protocols for transplanting the cells of the invention, the specification does not teach how to use the claimed methods to produce a therapeutic effect. The characteristics of the cells used in the claimed methods cannot make up for this deficiency" (Office action of October 12, 1999, Paper 36, pg. 5) Applicants respectfully disagree.

The characteristics of the cells are ***fundamentally*** what produce the therapeutic effect when transplanted into the recipient. The specification discloses that:

> It is well recognized in the art that transplantation of tissue into the CNS offers the potential for treatment of neurodegenerative disorders and CNS damage due to injury (review: Lindvall, (1991) Tins 14(8): 376-383). Transplantation of new cells into the damaged CNS has the potential to repair damaged circuitries and provide neurotransmitters thereby restoring neurological function. However, the absence of suitable cells for transplantation purposes has prevented the full potential of this procedure from being met. "Suitable" cells are cells that meet the following criteria: 1) can be obtained in large numbers; 2) can be proliferated *in vitro* to allow insertion of genetic material, if necessary; 3) capable of surviving indefinitely but stop growing after transplantation to the brain; 4) are non-immunogenic, preferably obtained from a patient's own tissue; 5) are able to form normal neural connections and respond to neural physiological signals (Bjorkiund (1991) Tins 14(8): 3 19-322). The progeny of multipotent neural stem cells obtainable from embryonic or adult CNS tissue, which are able to divide indefinitely when maintained *in vitro* using the culture conditions described herein, meet all of the desirable requirements of cells suitable for neural transplantation purposes and are a particularly suitable cell line as the cells have not been immortalized and are not of tumorigenic origin. The use of multipotent neural stem cells in the treatment of neurological disorders and CNS damage can be demonstrated by the use of animal

models.

See, specification pg. 26, lines 12-31. Note that the specification describes what was "well-known in the art" in 1991. Recent advances in neural stem cell research have since confirmed that characteristics of the cells are what was well-recognized in 1991.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), the named inventors and their co-workers have published numerous papers reporting results of transplantation of CNS neural stem cells. Reynolds & Weiss, 255 Science 1707-1710 (1992) ("*Reynolds & Weiss*" (named inventors)) (submitted in the Information Disclosure Statement of May 23, 1997; reference 66) specifically mentions the use of CNS neural stem cells in experimental and therapeutic transplantation (*see, Reynolds & Weiss,* pg. 1709, right column). Dr. Baetge, also a named inventor (695 Ann N.Y. Acad. Sci. 285-291 (1993) ("*Baetge*") reviews the desired qualities of an "ideal" cell for nervous system transplantation. *Baetge* indicates that CNS neural stem cells possess these ideal qualities, since they can be expanded to form large quantities of cells, they are non-immortalized and thus non-tumorigenic, and capable of differentiating into neurons and glia (*see, e.g., Baetge*, pg. 286).

As also shown in the Amendment filed July 21, 1999 (Paper No. 35), Dr. Hammang (another named inventor) and co-workers reported several methods of transplanting CNS neural stem cells and the results of those transplantations. *See,* Hammang *et al.*, in 21 *Methods in Neurosciences*, 281-293 (Flanagan *et al.*, eds., Academic Press, San Diego, 1994) ("*Hammang I*"). *Hammang I* discloses the formation of myelinating oligodendrocytes *in vivo* from transplanted CNS neural stem cells, as well as the formation of various neural cell progeny from transplanted CNS neural stem cells that carry the genetic marker *lac*Z.

As further shown in the Amendment filed July 21, 1999 (Paper No. 35), additional evidence by the inventors shows that when the undifferentiated CNS neural stem cells are injected into the myelin-deficient rat spinal cord, they respond to endogenous cues within the mutant CNS and differentiate into myelinating oligodendrocytes. Hammang *et al.,* 147(1) Exp Neurol. 84-95 (1997) (*Hammang II*). *Hammang II* shows that, because CNS neural stem cells "are influenced to divide using growth factors, rather than oncogenes, and because they appear to make appropriate lineage decisions when transplanted into a mutant environment, they may provide an excellent

OX

source of cells for a variety of future therapies using cellular transplantation." (*see, Hammang II,* Abstract).

In summary, the specification shows that the characteristics of CNS neural stem cells is such that the specification enables ***how to use*** the claimed methods of transplantation of CNS neural stem cells for a therapeutic use. Accordingly, Applicants respectfully request that this rejection under 35 U.S.C. § 112, first paragraph, be withdrawn.

Scientific references.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), the state of the art at the time of filing was such that transplantation of neural cells generally was enabled. McKay, 276 Science 66-71 (1997) ("*McKay*") teaches that transplantation of CNS neural stem cells into a host ***had been done*** by 1997, using methods similar to the methods described in the specification (*see,* the many references in *McKay*). Note that *McKay* cites the inventors, Reynolds and Weiss, and much of their subsequent work with CNS neural stem cells (*see, e.g., McKay,* pg. 67, right column). In response, the Examiner alleges that "despite the evidence of *McKay*", the methods described in the specification have not been used to produce any therapeutic effect. Applicants respectfully disagree.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), the Applicants have provided scientific references that show transplantation of both mouse and human CNS neural stem cell using the methods of the invention for a therapeutic benefit.

Milward.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), Milward *et al.*, 50 J. Neurosci. Res. 862-871 (1997) ("*Milward*") successfully transplanted canine CNS neural stem cells both into rat and into a shaking (sh) pup myelin mutant dog (a model of human myelin diseases). However, the Examiner alleges that *Milward* has not demonstrate application of the claimed method to produce a therapeutic effect. Applicants respectfully disagree.

Milward transplanted canine neurospheres into the myelin-deficient (*md*) rat spinal cord, resulting in the production of myelin by graft-derived cells (*see, Milward,* pg. 868, col. 1, 2nd para.) Thus, *Milward* showed that transplanted CNS neural stem cells can differentiate in the recipient to form myelin-producing oligodendrocytes and therapeutically provide myelin to recipients (such as these myelin-deficient (*md*) rats) in need thereof.

Also regarding canine CNS neural Zhang *et al.*, 54 J. Neurosci. Res. 181-190 (1998)) ("*Zhang*", previously submitted with the Amendment filed July 21, 1999 (Paper No. 35)) shows that:

> Two weeks after transplantation, a white streak of average 4.5 mm (up to 8 mm) in length was seen along the dorsal cord of the *md* rat, an area that is otherwise semitranslucent because of lack of myelin (Fig. 4b). When the cells are injected at two separate sites, a longer (10-14 mm) white streaks or separate streaks were observed (Fig 4c). After the cord was stained with X-gal, the white streaks turned blue (Fig. 4c,c). A traverse section through the transplanted site indicated that the blue staining was localized mainly in the dorsal funiculus of the spinal cord. Immunostaining of the transplanted cells were proteolipid protein (PLP) positive (Fig. 4d) as well as MBP positive (data not shown). The host spinal cord is negative for PLP because of a mutation in the PLP gene (Duncan, 1995). Phenylenediamine-stained semithin sections confirmed that the "blue" cells were interspersed among the myelin sheaths (Fig. 4e). Similar results were obtained when oligospheres of passage 4, 8 and 16 were transplanted into the *md* rats.

See, Zhang, pp. 186-187. Thus, *Zhang* showed that transplanted CNS neural stem cells can differentiate in the recipient to form myelin-producing oligodendrocytes and therapeutically provide myelin to recipients (such as these myelin-deficient (*md*) rats) in need thereof.

<u>*Zigova & Sanberg; Flax; and Brüstle.*</u>

As shown in the Amendment filed July 21, 1999 (Paper No. 35), others skilled in the art have used the claimed methods of this invention to show therapeutically effective transplantation of human CNS neural stem cells into mice. Zigova & Sanberg, 16 Nature Biotechnol. 1007-1008 (1998) ("*Zigova & Sanberg*") is a scientific news article that critically reviews results showing that human CNS neural stem cells can, using the appropriate *in vivo* developmental cues, can differentiate to functional neural cells, even in a mouse brain. Flax *et al.*, 16 Nature Biotechnol. 1033-1039 (1998) ("*Flax*") describes the transplantation of human neural stem cells into mice to replace deficient mouse neuronal populations and "provides strong evidence that the NSCs [neural stem cells] are able to perform *in vivo* and *in vivo* all the critical functions previously described for their rodent counterparts" (*Zigova & Sanberg*, pg. 1007, middle column). Brüstle *et al.*, 16 Nature Biotechnol. 1040-1044 (1998) ("*Brüstle*") describes the implantation of fetal human CNS progenitor cells into mice that "acquire an oligodendroglial phenotype and participate in the myelination of host axons" (*Zigova & Sanberg*, pg. 1008, right column).

OX

The Examiner alleges that none of the references (*Zigova & Sanberg, Flax, and Brüstle*) describe a therapeutic benefit to the mice upon transplantation. Applicants respectfully disagree.

Flax showed that transplantation of CNS neural stem cells provides a therapeutic benefit in the meander tail (*mea*) mouse, a mouse mutant model of neurodegeneration and impaired development that is characterized by a cell-autonomous failure of granule neurons to develop or survive in the cerebellum, especially the anterior lobe. *Flax* transplanted human CNS neuralstem cells into newborn *mea* cerebella and confirmed that the human neural stem cells provided "replacement neurons" with the "definitive size, morphology, and location of cerebellar granule neurons (Fig. 6E-G)" (*Flax*, pg. 1037, col. 2,2nd para; reviewd by *Zigova & Sanberg*). Thus, *Flax* showed that transplanted CNS neural stem cells can differentiate in the recipient to form granule neurons and therapeutically provide replacement neurons to recipients (such as these *mea* mice) in need thereof.

Zhang.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), Zhang *et al*, 96 Proc. Natl. Acad. Sci. USA 4089-94 (1999) ("*Zhang*") showed that neural stem cell cultures were generated from both juvenile and adult rats and used to produce myelin-forming cells, and when transplanted into *md* rats, those cells produced "robust myelination".

Applicants note that the Examiner does not contradict or contravene the evidence of *Zhang*.

Yandava.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), Yandava *et al.*, 96(12) Proc. Natl. Acad. Sci. 7029-34 (1999) ("*Yandava*") showed that transplantation of CNS neural stem cells results in "global" cell replacement and therapeutically effective remyelination in mice. *Yandava* showed that clonal neural stem cells transplanted at birth-using a simple intracerebroventricular implantation technique-resulted in widespread engraftment throughout the dysmyelinated shiverer (*shi*) mouse brain with repletion of myelin basic protein (MBP). *Yandava* showed that a number of recipient animals evinced decrement in their symptomatic tremor. The Examiner admits that "some therapeutic effect was seen, in so far as a number recipient animals showed a decline in symptomatic tremor."

However, the Examiner alleges that "it is not evident that the neural stem cell clone (pg.

7030, column 1, paragraph 3) used in the experiment was derived in the same manner as is taught in the instant specification." Without agreeing with the Examiner on this issue, Applicants submit that Yandava does show that one skilled in the art cantransplant neural stem cells to a host for a therapeutic effect, *without undue experimentation.*

Fricker.

As shown in the Amendment filed July 21, 1999 (Paper No. 35), Fricker *et al.*, 19 J. Neurosci. 5990-6005 (1999) ("*Fricker*") showed that transplantation of CNS neural stem cells results in site-specific migration and neuronal differentiation of human neural progenitor cells after transplantation in the adult rat brain.. *Fricker* obtained CNS neural stem cells from the embryonic human forebrain. When transplanted into neurogenic regions in the adult rat brain, the subventricular zone, and hippocampus, the *in vitro* propagated cells migrated specifically along the routes normally taken by the endogenous neuronal precursors: along the rostral migratory stream to the olfactory bulb and within the subgranular zone in the dentate gyrus, and exhibited site-specific neuronal differentiation in the granular and periglomerular layers of the bulb and in the dentate granular cell layer. The CNS neural stem cells exhibited substantial migration also within the non-neurogenic region, the striatum, and showed differentiation into both neuronal and glial phenotypes. Thus, *Fricker* showed the ability of the human neural stem cells to respond *in vivo* to guidance cues and signals that can direct their differentiation along multiple phenotypic pathways. *Fricker also* suggested that these cells can provide a powerful and virtually unlimited source of cells for experimental and clinical transplantation.

The Examiner has determined that "the in vivo behavior of CNS neural stem cells is not sufficient evidence to enable the methods of the invention, as the methods of the invention are clearly intended to provide a therapeutic benefit as evidenced by the specification." The Examiner's determination is "arbitrary." Administrative Procedure Act (APA), 5 U.S.C. § 706(2)(A). The Examiner *arbitrarily* ignores the evidence of the specification as a whole and of the teachings scientific literature in favor of an incorrect reading of that scientific literature and an improper standard of enablement.

The specification enables the claims for the "method of transplanting CNS neural stem cell progeny to a host comprising: transplanting one or more central nervous system (CNS) neural stem cells to said host." One of ordinary skill in the neurobiological art could readily have, at the

filing date, transplanted the claimed CNS neural stem cells into a host without undue experimentation. Accordingly Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

CONCLUSION

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

John Prince

Ivor R. Elrifi, Reg. No. 39,529
John Prince, Reg. No. 43,019
Attorneys for Applicants
c/o MINTZ LEVIN
One Financial Center
Boston, Massachusetts 02111
Telephone: (617) 542-6000
Telefax: (617) 542-2241

TRADOCS:1278363.1(R##301!.DOC)
04/12/00 3:07 PM